# MATERNAL TOBACCO SMOKE EXPOSURE CAUSES SEX-DIVERGENT CHANGES IN PPARγ AND WNT SIGNALING IN THE LUNG OF NEWBORN RAT PUPS

by

Alyssa Michelle Cox

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# STATEMENT OF THESIS APPROVAL

The thesis of **Alyssa Michelle Cox**

has been approved by the following supervisory committee members:

| | | |
|---|---|---|
| **Lisa Joss-Moore** | , Chair | **3/9/17** Date Approved |
| **Thunder Jalili** | , Member | **3/9/17** Date Approved |
| **Joan Benson** | , Member | **3/9/17** Date Approved |

and by **Scott Summers** , Chair of

the Department of **Nutrition and Integrative Physiology**

and by David B. Kieda, Dean of The Graduate School.

# ABSTRACT

Fetal exposure to maternal tobacco smoke (MTS) causes growth restriction and alters lung development in humans and animal models. Lung development depends upon molecular mechanisms including PPARγ and Wnt signaling. Recent reports suggest that chromatin-mediated negative cross-talk between PPARγ and Wnt signaling reduces transcription of Wnt output genes. This chromatin-mediated cross-talk is initiated by the lysine histone methyltransferase, and PPARγ target gene, Setd8. We previously showed that lung protein abundance of PPARγ is decreased by MTS exposure in both male and female rat pups. However, the effect of MTS exposure on Setd8 abundance, Wnt signaling, and expression of Wnt target genes in the rat lung is unknown.

We hypothesize that MTS exposure decreases Setd8 protein abundance in newborn rat lung. We also hypothesize that MTS exposure decreases transcription of Wnt output genes, in the context of increased Wnt signaling, in newborn rat lung.

Pregnant rats were exposed to room air (Control) or tobacco smoke (MTS) from gestational day 11.5 to term (day 22.5). Newborn pups were euthanized and lung tissue collected. We used western blot to measure β-catenin and Setd8 protein abundance in newborn female and male rat pup lung. We used real-time RT-PCR to measure mRNA transcript levels of Lef1, Tcf4, Axin2, MMP-9, and MMP-12 in newborn female and male rat lung.

Setd8 protein abundance did not change in MTS exposed female rat lung and was

significantly decreased in MTS exposed male rat lung compared to sex-matched controls. β-catenin protein abundance was increased in both female and male MTS exposed rat lung compared to sex-matched controls. mRNA transcript levels of Wnt transducer Lef1 were unchanged in MTS exposed female and male newborn rat lung compared to sex-matched controls. Tcf4 mRNA transcript levels significantly increased in MTS exposed female and male newborn rat pup lung compared to sex-matched controls. Axin2 mRNA transcript levels significantly decreased in only female newborn rat lung, while male Axin2 mRNA transcript levels were unchanged compared to sex-matched controls. mRNA transcript levels of Wnt target gene MMP-9 were significantly decreased in MTS exposed female and male newborn rat lung compared to sex-matched controls. MMP-12 mRNA transcript levels were increased in both female and male newborn rat lung compared with sex-matched controls.

Our results demonstrated that MTS exposure causes changes in Setd8 protein abundance as well as mRNA transcript levels of Wnt transducers and target genes in newborn rat lung. We speculate that the interaction of MTS exposure, the PPARγ-Setd8-H4K20me$^{1}$ pathway, and Wnt signaling pathway contribute to these changes.

TABLE OF CONTENTS

LIST OF FIGURES

# ACKNOWLEDGEMENTS

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# INTRODUCTION

Maternal tobacco smoke (MTS) exposure impacts more than 13% of pregnancies nationwide according to data from the Pregnancy Risk Assessment and Monitoring System (PRAMS).[1] Rates across the country vary widely. In some states, such as West Virginia, PRAMS data suggest the number of women who report smoking in the last 3 months of pregnancy is as high as 22%.[1] Despite antismoking campaigns and widespread knowledge of its detriments, MTS exposure during gestation remains a serious public health problem.

MTS exposure has been linked to many negative effects on the developing fetus. Women who smoke during pregnancy are at increased risk for preterm birth as well as reduced birth weight and intrauterine growth restriction.[2] Even short-term exposure has been shown to have deleterious effects. Mice embryos that experienced a single MTS exposure for only 4 hours showed a significant increase in the frequency of DNA deletions.[3] These DNA deletions are implicated in carcinogenesis and inheritable diseases.[3] Relevant to this study, MTS exposure causes significant lung disease in offspring.

Maternal tobacco use contributes to programming of the fetal lung directly and via effects on the placenta. While MTS exposure has immediate effects on the lung of the fetus, it is the additional programing of long-term effects that poses a significant public health risk.[4] Evidence supports the link between MTS exposure and impaired lung

functioning in offspring including increased wheezing, childhood asthma, and chronic obstructive pulmonary disease (COPD) later in life. [2,4] Impaired lung functioning and disease is often associated with altered lung structure.

The lung develops in distinct stages. The most relevant stage of lung development to MTS exposure is the last stage; that is alveolar formation. In the human lung, the alveolar stage begins at approximately gestational week 32 and continues postnatally.[5,6] Babies of mothers who smoke during pregnancy are often born preterm as a result of impaired placental vascularization and nutrient transfer.[2] Therefore, the last stage of lung development must occur postnatally. Lung development in non-human mammals goes through the same stages of lung development, although with different timing relative to birth. In rats at term birth, the lung is still in the saccular stage of development. Alveolar formation occurs later at postnatal day 4 and continues through postnatal day 14.[7,8] Because of this timing, animal models, particularly rodent models, have been used extensively to study the effects of MTS exposure on lung development.

Negative effects on lung functioning and structure from MTS exposure have also been shown in animal models. Findings consistently show alterations in lung growth and the formation of alveoli.[9,10] In rat studies, MTS exposure results in fewer, larger alveoli, as well as airway remodeling.[11,12] Alveolar formation is affected by multiple developmental signaling pathways and gene expression interactions. Notably, for this paper, the focus is peroxisome proliferator activated gamma (PPARγ) signaling and wingless (Wnt) signaling.

PPARγ is a member of the nuclear receptor family of transcription factors and is activated by long-chain polyunsaturated fatty acids. It is well known that PPARγ is

important for lung development, especially alveolar formation.[13] PPARγ is necessary for the epithelial-mesenchymal interactions required for lung development and lung vascular integrity.[14] One means by which PPARγ signaling may contribute to alveolar formation is via its actions on the epigenetic modifier, Setd8. PPARγ binds to a PPAR response element in the Setd8 promotor and directly promotes Setd8 transcription.[15] Setd8 is a histone lysine methyltransferase that places a methyl group on lysine 20 (K20) of H4, resulting in the H4K20me$^1$ epigenetic mark.[16] The H4K20me$^1$ mark influences the transcription of target genes via increased histone methylation. Recent evidence has suggested that important target genes regulated by H4K20me$^1$ and Setd8 in non-lung tissues include the output genes from Wnt signaling.[17]

Wnt is fundamental in cell signaling, regulating processes from proliferation to differentiation.[18,19,20] Wnt signaling is critical in lung development, contributing to differentiation of cell types that lead to proper alveolar formation.[20] Previous studies have shown that isolated nicotine exposure leads to decreased PPARγ and increased Wnt signaling in alveoli.[4,21] In canonical Wnt signaling, Wnt ligands activate a Wnt receptor and subsequently, β-catenin accumulates in the cytoplasm and is then translocated to the nucleus (Figure 1).[18,19] In the nucleus, β-catenin binds with T-cell factor/lymphoid enhancing factor (TCF/LEF) transcription factors (Figure 1).[18,19] This binding results in the transcription of Wnt target genes. Three important Wnt target genes examined in this study are Axin2, matrix metallopeptidase 9 and 12 (MMP-9 and MMP-12). Axin2 is a direct target of the Wnt pathway and acts as part of a negative feedback loop of the signaling pathway.[22] MMP-9 and MMP-12 are integral in lung structure development, promoting proper lung and alveolar formation.[23]

The point at which PPARγ and Wnt signaling converge occurs at the level of the chromatin and has been demonstrated in non-lung tissue. In response to Wnt activation, Setd8 is recruited to the β-catenin/TCF complex where it functions as a co-factor of transcription (Figure 1).[24] Recruitment of Setd8 enhances the H4K20me[1] mark in the region of the TCF binding element and promotes transcription of Wnt target genes.[24]

We previously showed that lung structure and function is altered in MTS exposed rat pups. We also showed that lung protein abundance of PPARγ is decreased by MTS exposure in both male and female rat pups.[1] However, whether MTS exposure affects Setd8 protein abundance, Wnt signaling, or expression of Wnt target genes in male and female newborn rat pup lung is unknown.

We hypothesize that MTS exposure decreases Setd8 protein abundance in newborn rat lung. We also hypothesize that MTS exposure decreases transcription of Wnt output genes, in the context of increased Wnt signaling, in newborn rat lung.

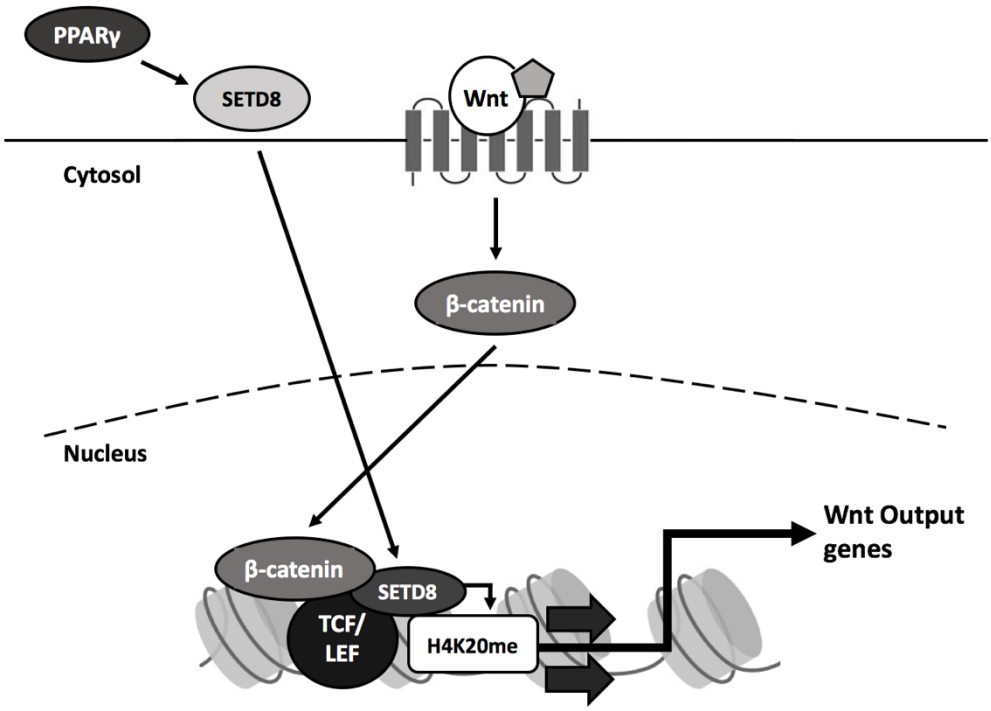


Figure 1. Model of Wnt signaling including Setd8 and β-catenin/TCF complex and its role in methylation and gene transcription.

# METHODS

## *MTS Rat Model*

Sprague-Dawley rat dams were exposed to tobacco smoke in a TE-10 Smoke Exposure System (Teague Enterprises, Davis, CA) using research grade cigarettes (Lexington, KY) as previously described by our lab.[25] Pregnant rats were exposed to tobacco smoke from gestational day 11.5 to term (gestational day 21.5). Determination of exposure was based on serum cotinine levels, a metabolite of nicotine. Neonatal cotinine ranges fell in the range indicating the mother as an "active smoker."[25] Control rats were housed in a similar manner, but were not exposed to tobacco smoke. At term delivery, rat pups were euthanized, lung tissue was harvested, and flash frozen in liquid nitrogen. Lung tissue was then stored at -80 degrees C.

We examined 4 groups for this study; MTS female, MTS male, control female, and control male. Each group consisted of n=6 rat pups derived from different litters.

## *Western Blot*

Western blotting was used to measure protein abundance with techniques previously described by our lab.[13,26] Briefly, total protein was extracted from newborn rat lung homogenate using RIPA buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.5% Na-deoxy-cholate, 1% NP- 40 (Igepal), and 0.1% SDS) and protease inhibitor (PI) cocktail (Roche-Complete Mini). Pierce BCA protein assay kit (ThermoScientific) was used to

generate a standard curve and measure protein concentration within each sample. Samples were stored at -80 degrees C until use

Western blot was used to measure abundance of Setd8 and β-catenin protein abundance in female and male MTS and control newborn rat lungs. The following antibodies were used: Setd8 (ab3798, Abcam) β-catenin (6B3, Cell Signaling Technology), and Anti Rabbit IgG HRP-linked (#7074S, Cell Signaling Technology). GAPDH was used as an internal control (14C10, Cell Signaling Technology). We loaded 10-15 micrograms of lung homogenate on an 8% Bis-Tris polyacrylamide midi gel in a MOPS buffer for electrophoresis. Following electrophoresis for 1 hour at 140 V, the gel was transferred to a polyvinyl membrane at 100 V for 1 hour while on ice. The blocking agent was 5% milk, the washing solution was TBS-T1%, and the overnight protocol was used on a Blot Cycler. Antibodies were detected with Western Lightning enhanced chemiluminescence and quantified using an Image Station 2000R (Eastman Kodak).

### *Real-Time RT-PCR*

Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was used to measure mRNA transcript levels of Wnt transduction genes Tcf4 and Lef1, as well as Wnt output genes Axin2, MMP-9, and MMP-12 in MTS exposed and control newborn rat pup lung tissue. RNA was extracted from lung tissue samples using an RNeasy Mini Kit (Qiagen). cDNA was generated using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The following Assay-on demand primer/probe sets were used: Tcf4-Rn00584481_m1; Lef1-Rn01522501_m1; Axin2-Rn00577441_m1; MMP-9-Rn00579162_m1; and MMP-12- Rn00588640_m1. GAPDH was used as an internal

control (GAPDH primer and probe sequences. Forward: CAAGATGGTGAAGGTCGGTGT; Reverse: CAAGAGAAGGCAGCCCTGGT; Probe: GCGTCCGATACGGCCAAATCCG). Data and RT-PCR amplification were analyzed by QuantiStudio 12K Flex Real Time PCR system.

### *Statistical Analysis*

Data are expressed as the mean ± standard deviation (SD). ANOVA with Fisher's least-significant-difference test was used to detect statistically significant differences between study groups. Using the StatView 5 software package (SAS Institute, Inc.), statistical significance was accepted at $p \leq 0.05$

# RESULTS

## *Setd8*

Because we previously observed a decrease in lung PPARγ protein abundance in MTS exposed female and male rat pups, we first examined Setd8 protein abundance relative to GAPDH in newborn rat pup lung. We determined 1) basal sex differences in Setd8 protein abundance in control rat lung, and 2) the effect of MTS exposure on Setd8 protein abundance in female and male rat lung. Setd8 protein abundance was significantly lower in female control than male control newborn rat pup lung ($p<0.05$). MTS exposure did not affect female newborn rat pup lung Setd8 protein abundance, but did significantly decrease male newborn rat pup lung Setd8 levels compared to sex-matched controls ($p<0.05$) (Figure 2).

## *Wnt Signaling Pathway*

In order to assess Wnt signaling, we measured β-catenin protein abundance relative to GAPDH in newborn rat pup lung. We determined 1) basal sex differences in β-catenin protein abundance in control rat lung, and 2) the effect of MTS exposure on β-catenin protein abundance in female and male rat lung. β-catenin protein abundance was similar in both female control and male control newborn rat pup lung. MTS exposure increased β-catenin protein abundance in both female ($p<0.05$) and male ($p<0.05$) newborn rat pup lung compared to sex-matched controls (Figure 3).

To further assess Wnt signaling, we examined mRNA transcript levels of Wnt transduction genes Tcf4 and Lef1 in newborn rat pup lung. We determined 1) basal sex differences in mRNA transcript levels in control rat lung, and 2) the effect of MTS exposure on mRNA transcript levels in female and male rat lung. Tcf4 mRNA transcript levels were similar in both female control and male control newborn rat pup lung. MTS exposure increased Tcf4 mRNA transcript levels relative to GAPDH in both female ($p<0.05$) and male ($p<0.05$) newborn rat pup lung compared to sex-matched controls (Figure 4). Lef1 mRNA transcript levels were similar in both female control and male control rat pup lung. MTS exposure did not change Lef1 mRNA transcript levels relative to GAPDH in either female or male newborn rat pup lung compared to sex-matched controls (Figure 5).

### *Wnt Target Output Genes*

To assess possible interface between Setd8, Wnt signaling, and Wnt target gene output, we measured mRNA transcript levels of Wnt output genes Axin2, MMP-9, and MMP-12 in newborn rat pup lung. We determined 1) basal sex differences in mRNA transcript levels in control rat lung, and 2) the effect of MTS exposure on mRNA transcript levels in female and male rat lung. Axin2 mRNA transcript levels were significantly higher in female control than male control newborn rat pup lung ($p<0.05$). MTS exposure significantly decreased Axin2 mRNA transcript levels relative to GAPDH in female ($p<0.05$), but not male newborn rat pup lung compared to sex-matched controls (Figure 6).

Next, we tested MMP-9 mRNA transcript levels. MMP-9 mRNA transcript levels

were significantly higher in female control than male control newborn rat pup lung ($p<0.05$). MTS exposure significantly decreased MMP-9 mRNA transcript levels relative to GAPDH in both female ($p<0.05$) and male ($p<0.05$) newborn rat pup lung compared to sex-matched controls (Figure 7).

Finally, we measured MMP-12 mRNA transcript levels. MMP-12 mRNA transcript levels were too low to detect in male control newborn rat pup lung. MTS exposure significantly increased MMP-12 levels relative to GAPDH in both female ($p<0.05$) and male ($p<0.05$) newborn rat pup lung compared to sex-matched controls (Figure 8).

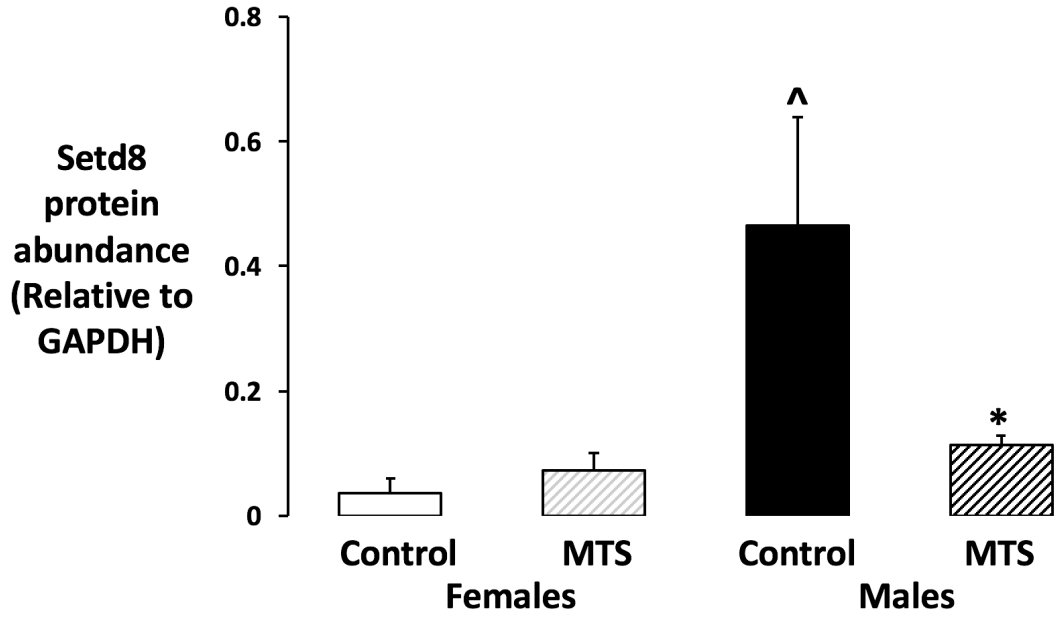


Figure 2. Setd8 protein abundance relative to GADPH
*$p<0.05$ compared to sex-matched control; ^$p<0.05$ compared to female control

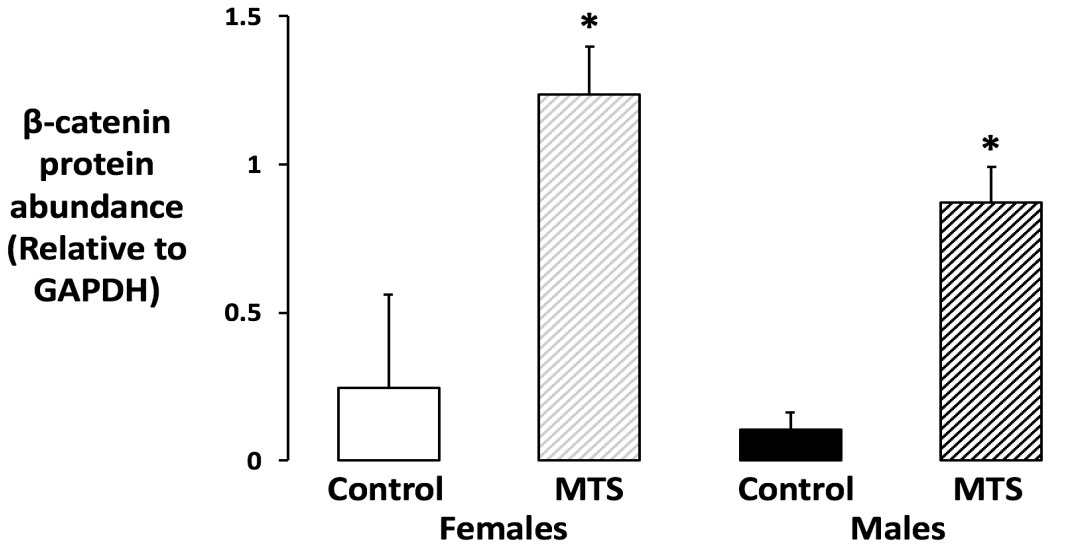


Figure 3. β-catenin protein abundance relative to GAPDH
*p<0.05 compared to sex-matched control

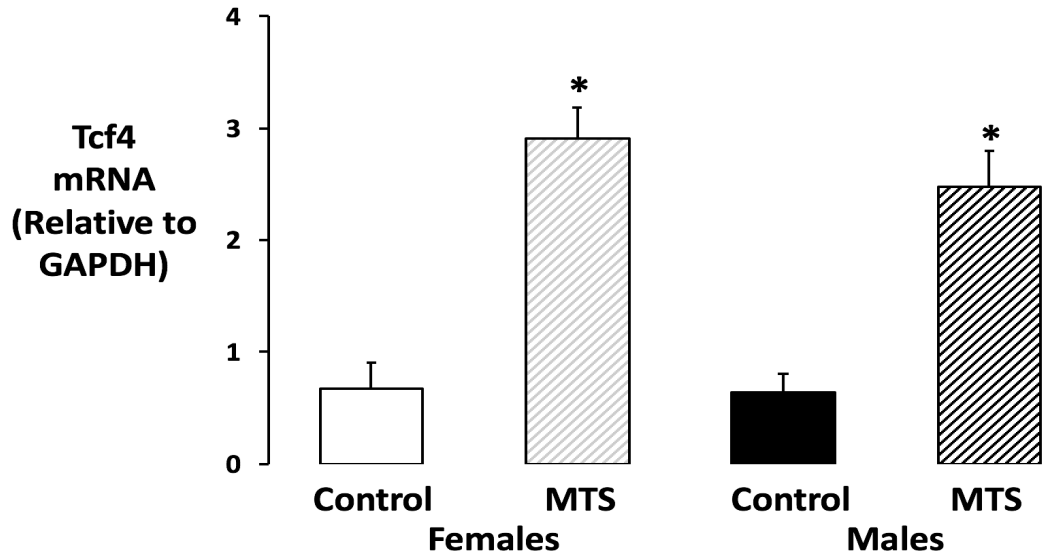


Figure 4. Tcf4 mRNA transcript levels relative to GAPDH
*p<0.05 compared to sex-matched control

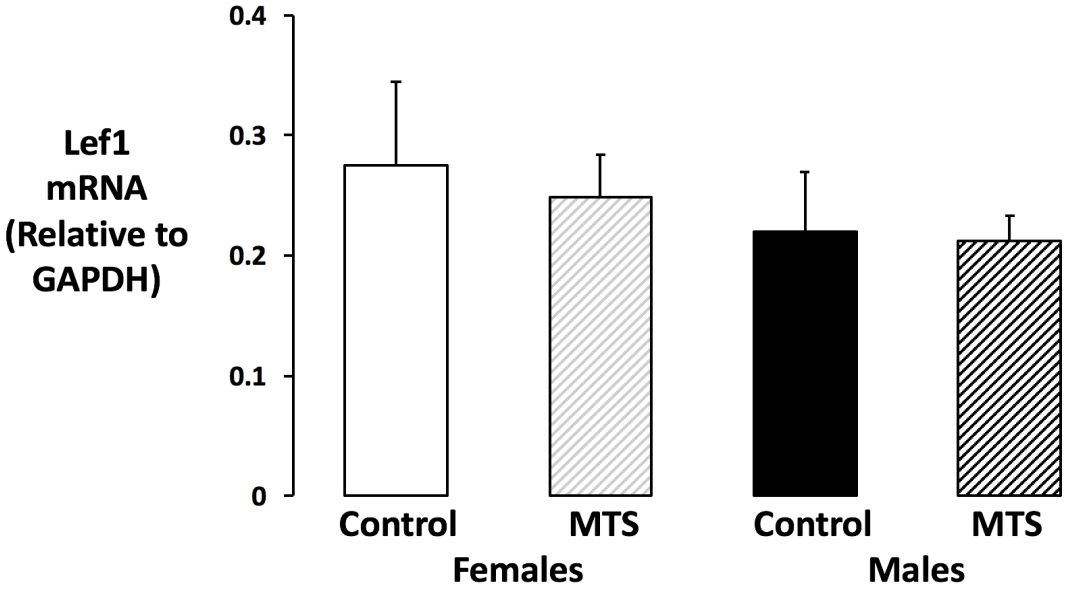


Figure 5. Lef1 mRNA transcript levels relative to GAPDH

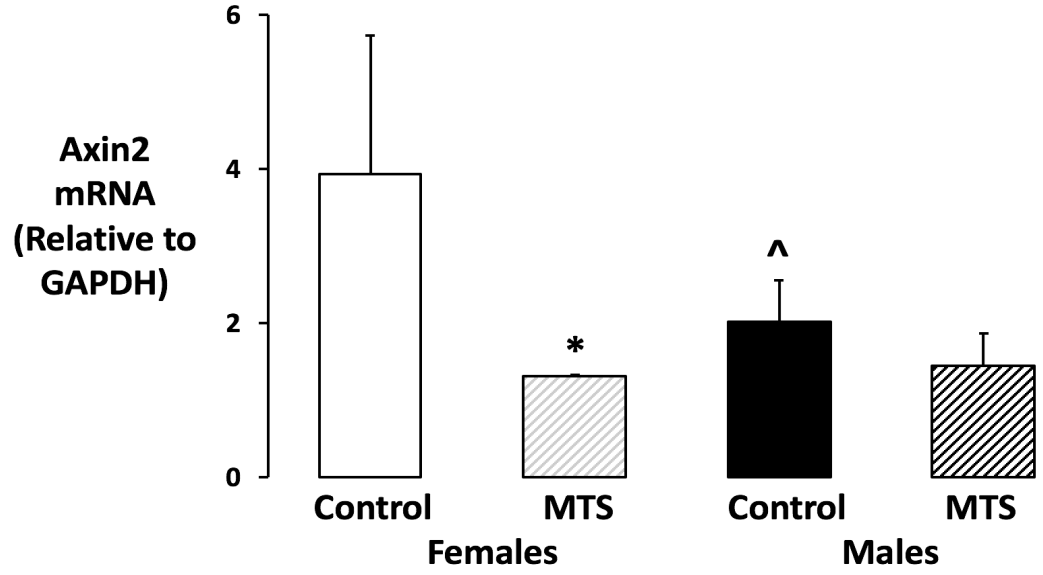


Figure 6. Axin2 mRNA transcript levels relative to GAPDH
*p<0.05 compared to sex-matched control; ^p<0.05 compared to female control

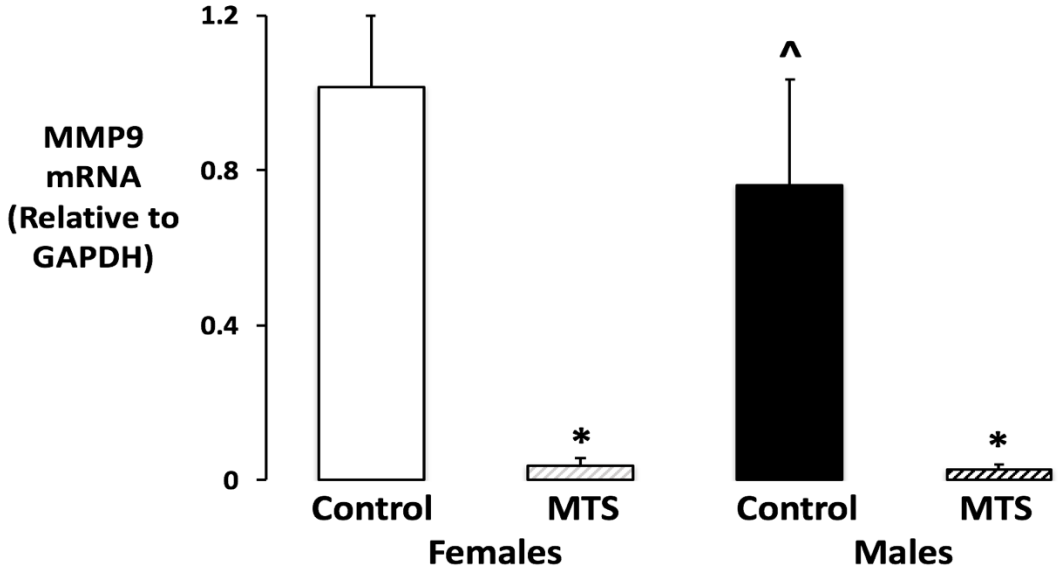


Figure 7. MMP-9 mRNA transcript levels relative to GAPDH
*p<0.05 compared to sex-matched control; ^p<0.05 compared to female control

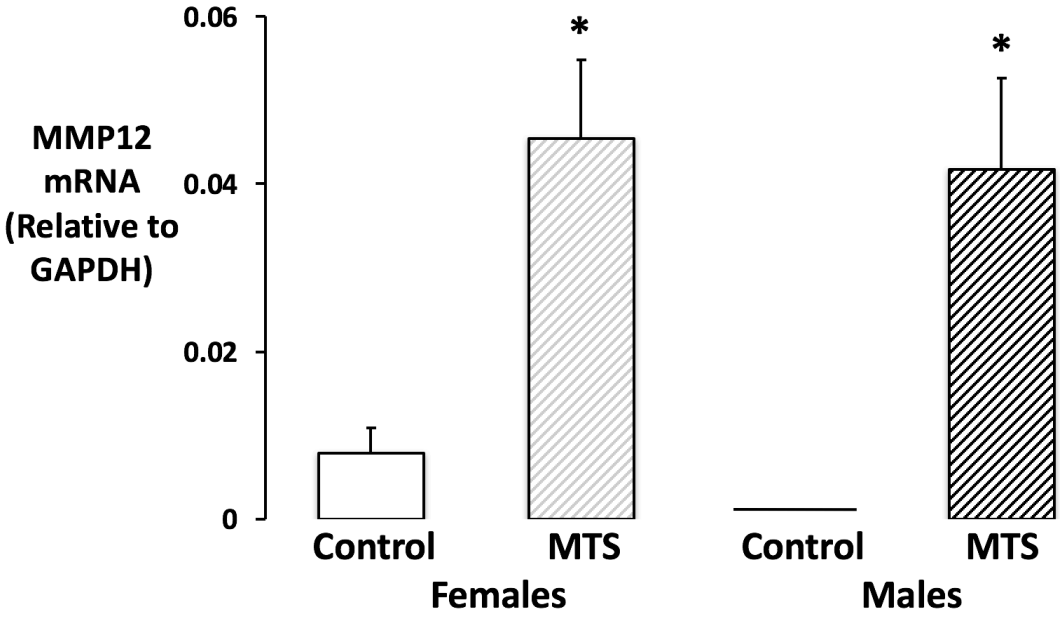


Figure 8. MMP-12 mRNA transcript levels relative to GAPDH
*p<0.05 compared to sex-matched control

# DISCUSSION

The results of our study demonstrate the impact of MTS exposure on molecular mechanisms of lung development. Our observations of increased lung Wnt signaling are consistent with previous reports. However, our data suggest that Wnt output gene expression is not always increased in the context of increased Wnt signaling. Deviations in Wnt target gene expression may be the result of deficient H4K20me[1] and Setd8 at the promotor region of Wnt target genes. Thus, our study suggests the interface between PPARγ and Wnt signaling may be disrupted in MTS exposed rat lungs.

β-catenin protein abundance, as well as Tcf4 mRNA transcript levels, were increased in MTS exposed female and male rat lung. Because of these elevated levels, we can assume that Wnt signaling was also increased in both MTS exposed female and male rat lung. Increased Wnt signaling in MTS exposed rat lung is consistent with previous reports.[21] Increases in Wnt signaling have been shown to be detrimental to lung development and function because Wnt signaling promotes transdifferentiation of pulmonary lipofibroblasts to myofibroblasts.[4] We speculate that increased Wnt signaling during lung development leads to persistent alterations in lung structure and function seen in older MTS exposed rats.

Our study showed sex differences in basal Setd8 protein abundance in our control female and male rat lung. Male control lung had significantly higher levels of Setd8 than female control lung. Additionally, MTS exposure caused sex-divergent changes in Setd8

protein abundance. Specifically, we found decreases in Setd8 protein abundance in male rat lung, but not female rat lung. We were not expecting a sex divergent difference in Setd8 protein abundance, as both female and male newborn MTS rat lungs have decreased PPARγ levels in previous research.[27] Male rat lung responded in the expected decreased manner, but it is possible that Setd8 expression is being regulated by something other than PPARγ in females. Estrogen has been shown to alter PPARγ signaling and could help explain the lower Setd8 protein abundance in female control rat lung as well as the lack of change in Setd8 protein abundance in female MTS exposed rat lung.[17,28]

In our study, we also found deviations in Wnt target gene expression. We hypothesized that MTS exposure would decrease Setd8 abundance and H4k20me$^1$. This would, in turn, decrease Wnt output gene expression of Axin2, MMP-9, and MMP-12 in both female and male rat lung. Our findings showed that MMP-9 mRNA transcript levels decreased in both female and male rat lung as we had expected. We speculate that the decrease in male rat lung is due to deficiencies in Setd8 and H4k20me$^1$ in the promotor regions of MMP-9. However, female rat lung also had decreased in MMP-9 without concomitant decreased Setd8. These results highlight the complexity in Wnt signaling output. Many studies of Wnt signaling in the lung assess only Wnt ligands, transduction components, and B-catenin without a complete examination of Wnt target gene expression. Studies that have examined the effects of MTS on target gene expression in the developing lung have shown that some target genes are downregulated.[29] To further understand the origin of decreased Wnt target gene expression in the context of increased Wnt signaling, H4K20me$^1$ levels and co-recruitment of Setd8 at target gene promoters

must be examined.

We have previously reported that Wnt output genes are not always affected in the expected way based on PPARγ and Setd8 changes.[17] Altered expression of Wnt target genes Axin2, MMP-9, and MMP-12 could be attributed to causes other than Setd8-mediated chromatin modifications. For example, cross talk between the Wnt signaling pathway and estrogen signaling is known to occur via a functional interaction between β-catenin and estrogen receptor alpha (ERα) in other tissues.[17] This interaction with estrogen could be affecting Wnt output gene expression and explain the sex-divergent results we see in newborn rat lung.

Additionally, it is possible that specific Wnt output genes must be expressed at a given developmental time point for proper lung development and alveolar formation. Axin2, MMP-9, MMP-12 are each relevant in immature lungs. As previously stated, Axin2 acts as a negative regulator of the Wnt signaling pathway, while MMP-9 and MMP-12 are vital in lung structure development and promote proper lung and alveolar formation.[22,23] We studied only newborn rat lung so it is possible that these Wnt target gene outputs would be expressed at altered levels in an adolescent or adult lung that are no longer developing. Furthermore, basal differences seen in mRNA transcript levels that we measured in control females and males could be due to differences in developmental timing between females and males.

Our study is not without limitations. At present, we do not know H4K20me$^1$ levels at the promotors of the Wnt output genes measured. Our group is currently conducting research using chromatin immunoprecipitation (ChIP) to measure H4K20me$^1$ at the promotor regions of Axin2, MMP-9, and MMP-12. Because Setd8 levels were

decreased in male rat lung, we speculate that H4K20me$^1$ will be decreased at the promotor regions of these Wnt output genes in male rat lung as well. Results of the ChIP-sequencing will further our knowledge of the interface between the PPARγ-Setd8-H4K20me$^1$ pathway and Wnt signaling pathway to better understand Wnt output gene expression.

Another limitation is that our study is descriptive in nature, i.e. we did not establish a cause-and-effect relationship between PPARγ-Setd8-H4K20me$^1$ and Wnt signaling output. Other studies by our group are assessing cause-and-effect relationships between PPARγ-Setd8-H4K20me$^1$ and Wnt signaling output using cell culture studies in vitro. Additionally, in vivo studies have been conducted by our group that manipulate the PPARγ pathway with dietary lipids to show causal relationships.[13] Specifically, they have shown that decreased PPARγ and Setd8 protein abundance due to intrauterine growth restriction were ameliorated with maternal supplementation of docosahexaenoic acid (DHA), a long-chain polyunsaturated fatty acid.[13] We speculate that maternal DHA supplementation would show similar effects in MTS exposed rat lung.

In conclusion, the PPARγ-Setd8-H4K20me$^1$ and Wnt signaling pathways are sex-dependent and are affected by MTS exposure in newborn rat lung. MTS exposure decreases Setd8 protein abundance in male newborn rat lung. MTS exposure increases Wnt signaling in both female and male newborn rat lung, and dysregulates Wnt target gene output. Our results provide the important first step in understanding effects of MTS exposure on the interaction between the PPARγ-Setd8-H4K20me$^1$ pathway and Wnt signaling pathway in vivo.

REFERENCES


1. (*PCH) Indicator for whether mother smoked during the three months before getting pregnant. https://nccd.cdc.gov/PRAMStat/rdPage.aspx?rdReport=DRH_PRAMS.ExploreByTopic&islClassId=CLA9&islTopicId=TOP27&go=GO. Accessed November 29, 2016.

2. McQueen CA. Environmental Exposures and Developmental Programming of the Lung. In: *Comprehensive Toxicology*. 3rd ed. Elsevier; 2010.

3. Jalili T, Murthy GG, Schiestl RH. Cigarette smoke induces DNA deletions in the mouse embryo. *Cancer Res*. 1998;58(12):2633-2638.

4. Rehan VK, Asotra K, Torday JS. The effects of smoking on the developing lung: insights from a biologic model for lung development, homeostasis, and repair. *Lung*. 2009;187(5):281-289. doi:10.1007/s00408-009-9158-2.

5. Albertine KH, Pysher TJ. Impaired lung growth after injury in premature lung. *Fetal and Neonatal Physiology*. Elsevier; 2003:942-949. doi:10.1016/B978-0-7216-9654-6.50096-5.

6. Burri PH. Structural aspects of postnatal lung development – alveolar formation and growth. *Neonatology*. 2006;89(4):313-322. doi:10.1159/000092868.

7. Burri PH, Dbaly J, Weibel ER. The postnatal growth of the rat lung. I. Morphometry. *Anat Rec*. 1974;178(4):711-730. doi:10.1002/ar.1091780405.

8. Herring MJ, Putney LF, Wyatt G, Finkbeiner WE, Hyde DM. Growth of alveoli during postnatal development in humans based on stereological estimation. *AJP Lung Cell Mol Physiol*. 2014;307(4):L338-L344. doi:10.1152/ajplung.00094.2014.

9. Rehan VK, Sakurai R, Wang Y, Santos J, Huynh K, Torday JS. Reversal of nicotine-induced alveolar lipofibroblast-to-myofibroblast transdifferentiation by stimulants of parathyroid hormone-related protein signaling. *Lung*. 2007;185(3):151-159. doi:10.1007/s00408-007-9007-0.

10. Rehan VK, Liu J, Naeem E, et al. Perinatal nicotine exposure induces asthma in second generation offspring. *BMC Med*. 2012;10:129. doi:10.1186/1741-7015-10-

129.

11. Collins MH, Moessinger AC, Kleinerman J, et al. Fetal lung hypoplasia associated with maternal smoking: a morphometric analysis. *Pediatr Res*. 1985;19(4):408-412.

12. Manoli SE, Smith LA, Vyhlidal CA, et al. Maternal smoking and the retinoid pathway in the developing lung. *Respir Res*. 2012;13:42. doi:10.1186/1465-9921-13-42.

13. Joss-Moore LA, Wang Y, Baack ML, et al. IUGR decreases PPARγ and SETD8 expression in neonatal rat lung and these effects are ameliorated by maternal DHA supplementation. *Early Hum Dev*. 2010;86(12):785-791. doi:10.1016/j.earlhumdev.2010.08.026.

14. Simon DM, Arikan MC, Srisuma S, et al. Epithelial cell PPAR[gamma] contributes to normal lung maturation. *FASEB J*. 2006;20(9):1507-1509. doi:10.1096/fj.05-5410fje.

15. Wakabayashi K -i., Okamura M, Tsutsumi S, et al. The peroxisome proliferator-activated receptor gamma/retinoid x receptor alpha heterodimer targets the histone modification enzyme PR-Set7/Setd8 gene and regulates adipogenesis through a positive feedback loop. *Mol Cell Biol*. 2009;29(13):3544-3555. doi:10.1128/MCB.01856-08.

16. Couture J-F, Collazo E, Brunzelle JS, Trievel RC. Structural and functional analysis of SET8, a histone H4 Lys-20 methyltransferase. *Genes Dev*. 2005;19(12):1455-1465. doi:10.1101/gad.1318405.

17. Ke X, Xing B, Yu B, et al. IUGR disrupts the PPARγ-Setd8-H4K20me(1) and Wnt signaling pathways in the juvenile rat hippocampus. *Int J Dev Neurosci*. 2014;38:59-67. doi:10.1016/j.ijdevneu.2014.07.008.

18. Morrisey EE, Cardoso W V., Lane RH, et al. Molecular determinants of lung development. *Ann Am Thorac Soc*. 2013;10(2):S12-S16. doi:10.1513/AnnalsATS.201207-036OT.

19. Minoo P, Li C. Cross-talk between transforming growth factor-β and Wingless/Int pathways in lung development and disease. *Int J Biochem Cell Biol*. 2010;42(6):809-812. doi:10.1016/j.biocel.2010.02.011.

20. Pongracz JE, Stockley RA. Wnt signalling in lung development and diseases. *Respir Res*. 2006;7(1):15. doi:10.1186/1465-9921-7-15.

21. Sakurai R, Cerny LM, Torday JS, Rehan VK. Mechanism for nicotine-induced up-regulation of Wnt signaling in human alveolar interstitial fibroblasts. *Exp Lung*

*Res*. 2011;37(3):144-154. doi:10.3109/01902148.2010.490288.

22. Jho E, Zhang T, Domon C, Joo C-K, Freund J-N, Costantini F. Wnt/beta-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. *Mol Cell Biol*. 2002;22(4):1172-1183. doi:10.1128/MCB.22.4.1172-1183.2002.

23. Logan CY, Nusse R. The wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol*. 2004;20(1):781-810. doi:10.1146/annurev.cellbio.20.010403.113126.

24. Li Z, Nie F, Wang S, Li L. Histone H4 Lys 20 monomethylation by histone methylase SET8 mediates Wnt target gene activation. *Proc Natl Acad Sci*. 2011;108(8):3116-3123. doi:10.1073/pnas.1009353108.

25. Zinkhan EK, Lang BY, Yu B, et al. Maternal tobacco smoke increased visceral adiposity and serum corticosterone levels in adult male rat offspring. *Pediatr Res*. 2014;76(1):17-23. doi:10.1038/pr.2014.58.

26. Joss-Moore L a, Wang Y, Yu X, et al. IUGR decreases elastin mRNA expression in the developing rat lung and alters elastin content and lung compliance in the mature rat lung. *Physiol Genomics*. 2011;43(9):499-505. doi:10.1152/physiolgenomics.00183.2010.

27. Joss-Moore L, Wang Y, Jiang C, et al. Maternal tobacco smoke exposure alters rat lung PPARγ and Setd8 abundance in a gender-specific manner through alveolarization. 2011.

28. Bonofiglio D, Gabriele S, Aquila S, et al. Estrogen receptor binds to peroxisome proliferator-activated receptor response element and negatively interferes with peroxisome proliferator-activated receptor signaling in breast cancer cells. *Clin Cancer Res*. 2005;11(17):6139-6147. doi:10.1158/1078-0432.CCR-04-2453.

29. Unachukwu U, Trischler J, Goldklang M, Xiao R, D'Armiento J. Maternal smoke exposure decreases mesenchymal proliferation and modulates Rho-GTPase-dependent actin cytoskeletal signaling in fetal lungs. *FASEB J*. February 2017:fj.201601063R. doi:10.1096/fj.201601063R.